

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

1/13

Constructs										pABV number	M-expression	N-expression
	5'UTR	ORF1ab	ORF2	ORF3	ORF4	ORF5	ORF6	ORF7	3'UTR	437	+	+
										594	-	+
										521	- <sup>b)</sup>	-
										664	-	+
										668	- <sup>b)</sup>	-

<sup>b)</sup> Identical results were obtained in IPMA using MAbs against GP<sub>3</sub> and GP<sub>4</sub>

Fig. 1A

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

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Docket No.: 2183-6217US

2/13

Constructs	Deletion (nucleotides)	PABV number	M- expression
	-	437	+ <sup>1)</sup>
	Δ 14588-14936	605	-
	Δ 14588-14885	604	-
	Δ 14588-14786	603	-
	Δ 14588-14687	602	-
	Δ 14588-14642	624	+
	Δ 14599-14642	625	+
	Δ 14588-14600	626	+ <sup>1)</sup>
	Δ 14938-14980	638	+ <sup>1)</sup>
	Δ 14887-14980	637	+
	Δ 14788-14980	636	+
	Δ 14686-14980	635	+
	Δ 14643-14686	631	-
	Δ 14643-14676	632	-
	Δ 14643-14664	633	-
	Δ 14643-14652	634	+
	Δ 14653-14686	696	-
	rescue of 696	730	+ <sup>1)</sup>

<sup>1)</sup> Identical results were obtained in IPMA using MAbs 122.17 against N

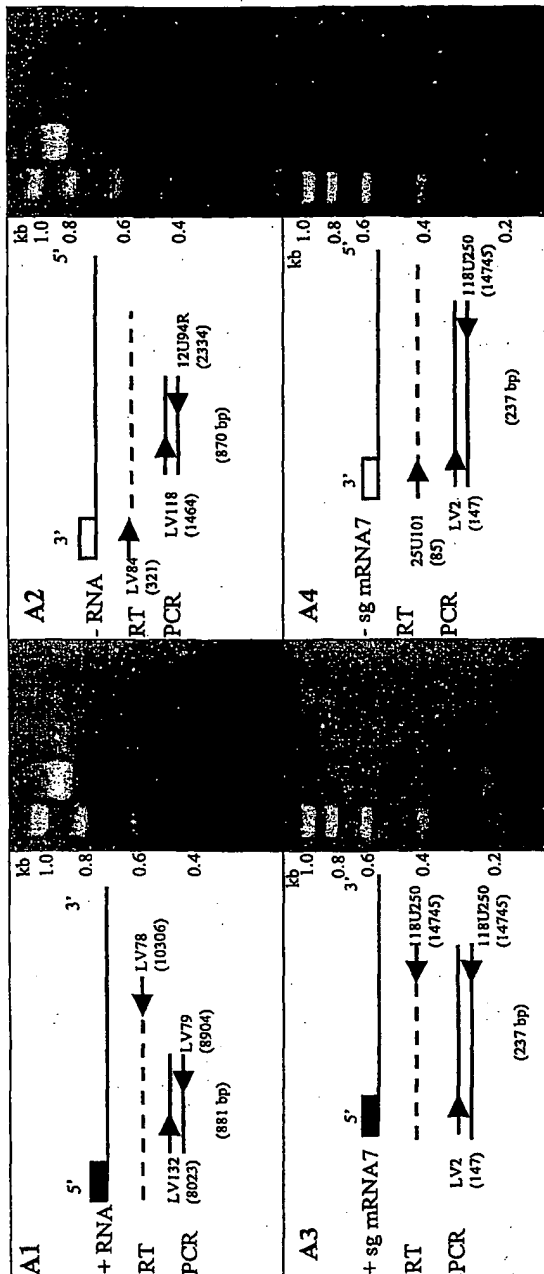
Fig. 1B

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

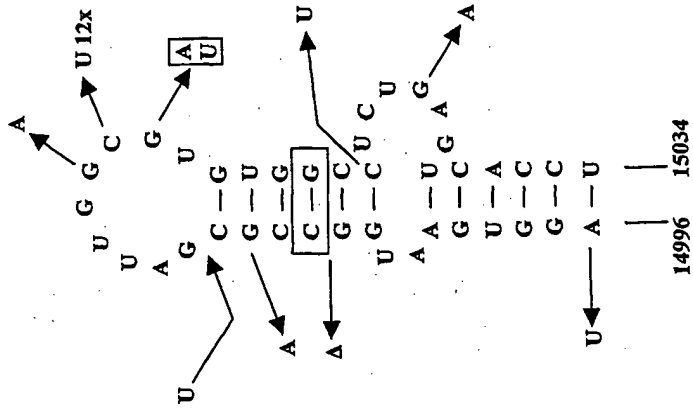
Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

3/13

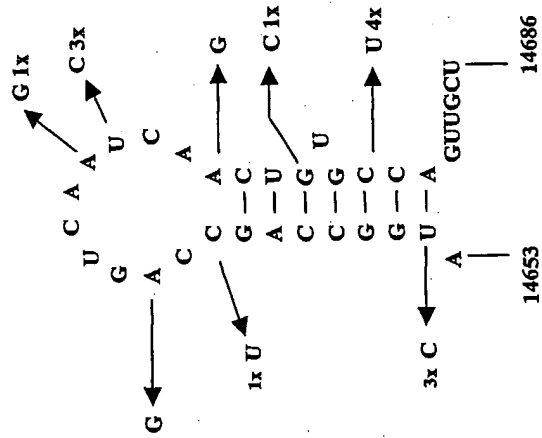
Fig. 2



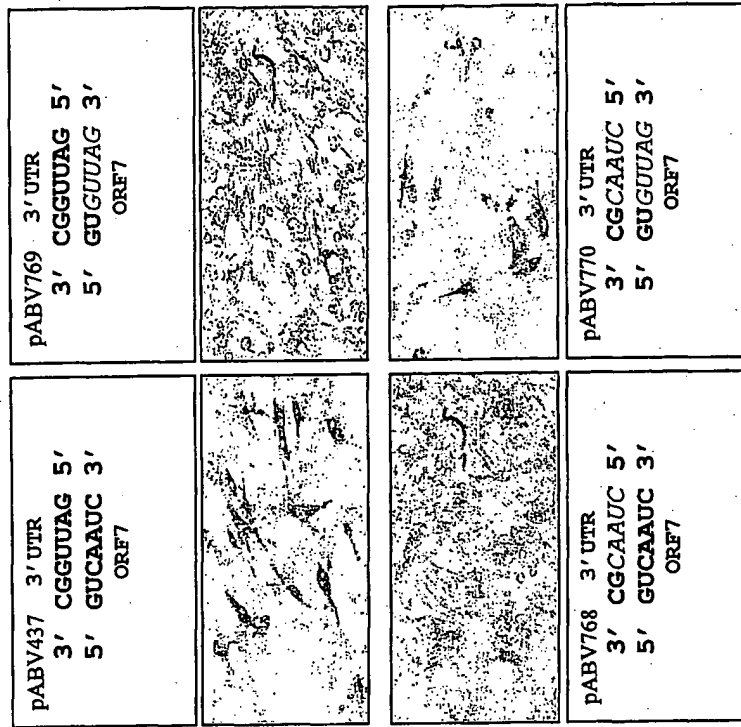
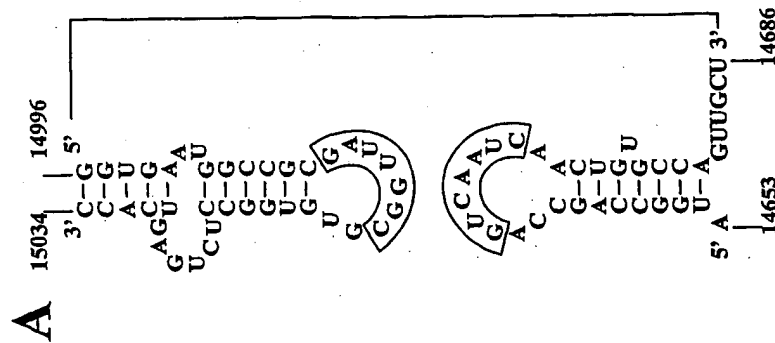
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**Fig. 3B**



**Fig. 3A**



**Fig. 4**

TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije  
Docket No.: 2183-6217US

6/13

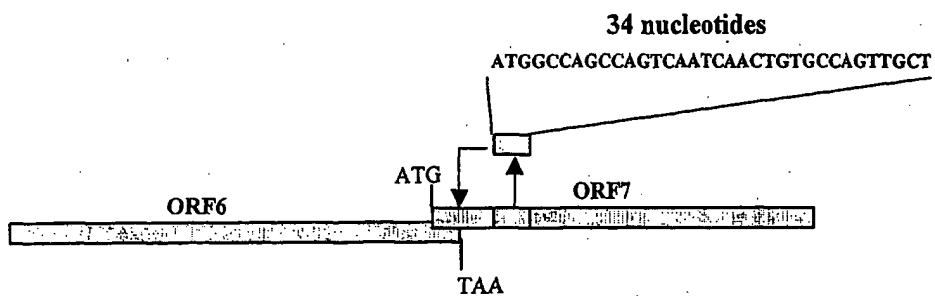


Fig. 5

7/13

A

LV : MAGKNSQKKKKSTAPMNGQPVNQLCOLLGAMIKSQRQ---QPRGGOAKKKKPEKPEPLAEDDIRHH : 67  
VR2332 : MPNNNGKQKKRK---KGDGQPVNQLCOLLGKIIAQQNQSRGKPGKRNKKKNPEKPEPLATEDDVRHH : 66  
\* \* \* \* \*  
\* \* \* \* \*

LV : LTQTERSLCLQSIQTAFNQAGTASLSSSGKVSFQVEFMLPVAHTVRLIRVTSTASQGAS : 128  
VR2332 : FTPSERQLCLSSSIQTAFNQAGTCTLSDSGRISYTYVEFSLPTHHTVRLIRVTASPSA---- : 123  
\* \* \* \* \*  
\* \* \* \* \*

B

LV : TTAACAGTCA-----GGTGAATGGCCCGGATTGGCG : 32  
VR2332 : TGGGCTGGCATCTTGGGCATCTCAGTGTGTTGAATTGGAAGAAATGTGTGTGAATGGCACTGATTGACA : 70  
\* \* \* \* \*  
\* \* \* \* \*

LV : TGTGGCCTCTGAGTCACCTATTCAATTAGGGCGATCACATGGGGGTCACTTAATCAGGCAGGAACCAT : 102  
VR2332 : TTGTGCCCTCTAAGTCACTTCAATTAGGGCGACCGTGTGGGGGTGAGATTTAATT-GGCGAGAACCAT : 139  
\* \* \* \* \*  
\* \* \* \* \*

LV : GTGACCGGAATTAAAAAAA: 122  
VR2332 : GCGGCCGGAATTAAAAAAA: 159  
\* \* \* \* \*

Fig. 6

TITLE: DELETIONS IN ARTERIVIRUS  
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Docket No.: 2183-6217US

8/13

Constructs	Deletion (nucleotides / amino acids)	Plasmid number	M- expression	N- expression	Virus production
	<p>wild type</p> <p>Δ14975-14980 / Δ 2</p> <p>Δ14969-14980 / Δ 4</p> <p>Δ14966-14980/ Δ 5</p> <p>Δ14963-14980/ Δ 6</p> <p>Δ14960-14980/ Δ 7</p> <p>Δ14957-14980/ Δ 8</p> <p>Δ14954-14980/ Δ 9</p> <p>Δ14989-14995</p> <p>Δ14989-15020</p>	<p>437</p> <p>639</p> <p>694</p> <p>745</p> <p>746</p> <p>747</p> <p>748</p> <p>695</p> <p>693</p> <p>729</p>	<p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>-</p>	<p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>-</p>	<p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>+</p> <p>-</p> <p>-</p> <p>-</p> <p>+</p> <p>-</p>

Fig. 7

9/13

Growth curves of PRRSV deletion mutants

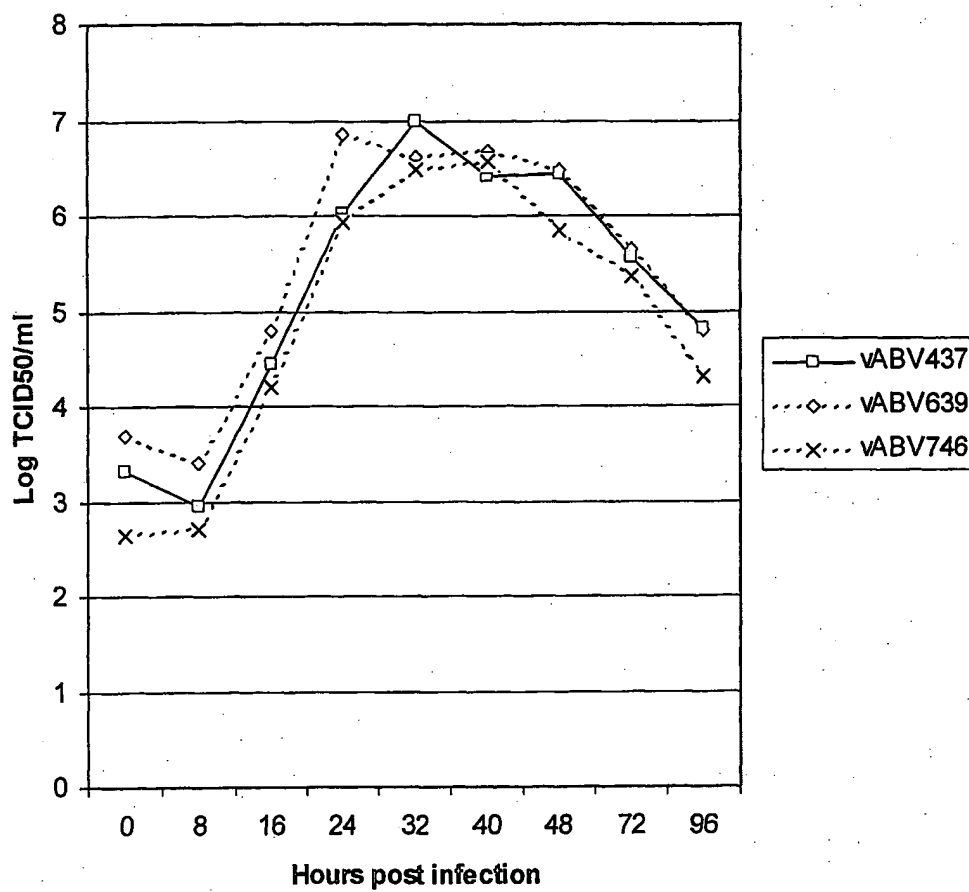


Fig. 8

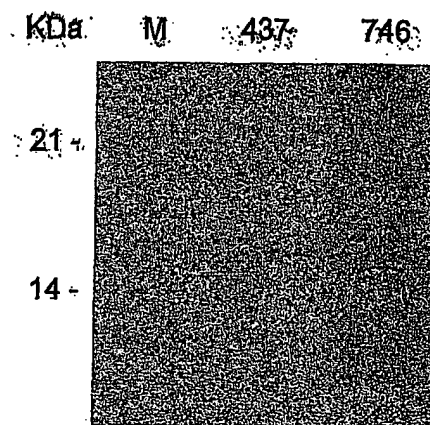
TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

10/13

Fig. 9



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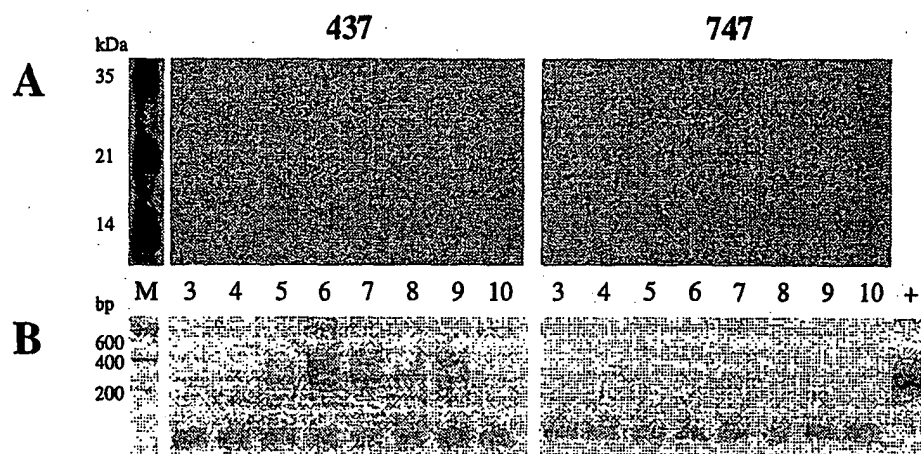
TITLE: DELETIONS IN ARTERIVIRUS  
REPLICONS

Inventor: Monique Helene Verheije

Docket No.: 2183-6217US

11/13

Fig. 10



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12/13

TABLE 1: Sequences of the primers used to introduce deletions by PCR, and primers used to sequence the introduced mutations.

Primer	Sequence of the primer <sup>a</sup>	Orientation	Purpose (pABV)	Location
119R218R	5' ATGACATCCGGCACCACC 3'	+	Sequencing	14782
LV20	5' CCTGATTAAAAAGCTTGACCCC 3'	-	Sequencing	15066
LV75	5' TCTAGGAATTCTAGACGATCG 3'	-	XbaI -site	15088
LV155	5' ACGTGCCTTAACCTCGTCAAGTATGGCCGGTAAAAACCAGAGCCAGA 3'	+	HpaI -site	14582
LV204	5' ACGTGCCTTAACCTCGTCAAGTATGGCCGGTATGTAGA 3'	-	639	14974
LV213	5' TGCAAGTTAATTAAAGGTGAATGGCCGCCGA 3'	+	693	14996
LV214	5' GACTGTTTAAATTAAGTGGCGGATGTA 3'	-	694	14958
LV215	5' GACTGTTTAAATTAAGTCAACGGAATC 3'	-	695	14942
LV239	5' TGCAAGTTAATTAAAGCCTCTGAGTCA 3'	+	729	15021
LV263	5' GACTGTTTAAATTAAGCGGATGTAGA 3'	-	745	14954
LV264	5' GACTGTTTAAATTAAGTGTAGAAATC 3'	-	746	14951
LV265	5' GACTGTTTAAATTAAGTAAAGTCACG 3'	-	747	14948
LV266	5' GACTGTTTAAATTAAGAAAGTCACGCCGA 3'	-	748	14945

<sup>a</sup> The restriction sites are underlined.

Fig. 11

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Docket No.: 2183-6217US

13/13

Fig. 12

TABLE 1: Sequences of the primers used to introduce deletions by PCR, primers used to sequence the introduced mutations, and primers used for the strand-specific RT-PCR

Primer	Sequence of the primer <sup>a</sup>	Orien- tation	Purpose (pABV)	Location
118U750	5' CAGCCAGGGGAAATGTGGC 3'	-	Sequencing / Strand-sp. PCR	14745
12U94R	5' CACCTGTACCTGCTCAATTGT 3'	-	Strand-sp. PCR	2334
23U101	5' GTTCTAGCCCAACAGGTATC 3'	+	Strand-sp. RT	85
L172	5' AGCGGGAAGGATCCACAGGTAT 3'	+	Strand-sp. PCR	147
L177	5' CCCTTGACGAGCTCTTCGGC 3'	+	Sequencing	14045
L1720	5' CCTGATTAAAGCTTGACCCC 3'	+	Sequencing	15066
L1775	5' TCTAGGAATCTAGACGATCG 3'	-	PCR XbaI-site	15088
L1776	5' TCTAGGAATCTAGACGATCG(T)40 3'	-	RT	15088
L1778	5' CCCTGGGATGAATCTATGTT 3'	-	Strand-sp. RT	10306
L1779	5' GACAAGATCATCAGAGTATACC 3'	-	Strand-sp. PCR	8904
L1784	5' AGAGCTTCAGGACACTGACC 3'	+	Strand-sp. RT	321
L1712	5' CCAITCACCTGACTGTITTAIAA	-	Strand-sp. PCR	14981
L17118	5' TTACCACTACTCTCCACCG 3'	+	PCR PacI-site	1464
L17132	5' CCTACTGTGCCTATAGTGT 3'	+	Strand-sp. PCR	8023
L17151	5' ACCAGGCGCAGAGAAAGAAAGTACAGCTGGGTGCAATGAT 3'	+	PCR (631)	14611
L17152	5' ACCAGGCGCAGAGAAAGAAAGTACAGCTGGGTGCTGG 3'	+	PCR (632)	14611
L17153	5' ACCAGGCGCAGAGAAAGAAAGTACAGCTTCAATCACTGT 3'	+	PCR (633)	14611
L17154	5' ACCAGGCGCAGAGAAAGAAAGTACAGCTATGGCCAGCCAG 3'	+	PCR (634)	14611
L17155	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	HpaI-site PCR	14582
L17188	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	PCR (602)	14582
L17189	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	PCR (603)	14582
L17190	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	PCR (604)	14582
L17191	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	PCR (605)	14582
L17195	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	PCR (624)	14582
L17196	5' GGAGTGGTAAACCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	PCR (625)	14582
L17197	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	+	PCR (626)	14582
L17198	5' GCTCGTGAAGCTTTAGCATCACATACAC 3'	+	NheI-site PCR	14140
L17200	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	-	PCR (635)	14981
L17201	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	-	PCR (636)	14981
L17202	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	-	PCR (637)	14981
L17203	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	-	PCR (638)	14981
L17204	5' ACGTGGCTTAAGCTCGTCAAGTATGGCGGTAAACACAGAGCCAGA 3'	-	PCR (639)	14981
L17216	5' ACCAGGCGCAGAGAAAGAAAGTACAGCTCCGATGGGGAG GGTCAATGAT 3'	+	PCR (696)	14611
L17268	5' ACCAGGCGCAGAGAAAGAAAGTACAGCTCCGATGGGGAG 3'	+	PCR (769)	14611
L17269	5' CTCGGATGGGAGGAGCCAGCGGTGTAAGAACTGTGCCAGT 3'	+	PCR (769)	14641
L17270	5' TGCAAGTTAATTAACAGTCAAGTGAATGGCCGCTAACCGGTGTGGCCTC 3' +	+	PCR (768)	14981

<sup>a</sup> The restriction sites are underlined.